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Enhancing cellular cancer vaccines

Edward P Cohen^{1,†}, Amla Chopra¹, InSug O-Sullivan¹, and Tae Sung Kim²

¹Department of Microbiology & Immunology (m/c 790), University of Illinois College of Medicine, 835 South Wolcott Ave, Chicago, IL 60612, USA

²School of Life Sciences & Biotechnology, Korea University, Seoul, Republic of Korea

Abstract

Various strategies have been used to generate cellular cancer vaccines with the expectation that they will become an effective part of the overall management of cancer patients. However, with few notable exceptions, immunization has not resulted in significant long-term therapeutic benefits. Tumor growth has continued and patient survival has been at best only modestly prolonged. One possible explanation is that as only a small proportion of the constituents of malignant cells are ‘tumor specific’ and the vast majority are the products of nonantigenic, normal ‘housekeeping’ genes, the immune response in patients immunized with cellular cancer vaccines is not sufficient to result in tumor rejection. Here, we review and characterize various types of cellular cancer vaccines. In addition, in a mouse breast cancer model system, we describe a unique strategy designed to enrich cellular vaccines for cells that induce tumor immunity. Numerous advantages and disadvantages of cancer immunotherapy with cellular vaccines are also presented.

Keywords

cancer vaccine; dendritic cell; dNA vaccine; enriched vaccine; fibroblast; leukapheresis; tumor dNA

Numerous attempts have been made to prepare effective cancer vaccines that can be used to treat cancer patients in the expectation that the anti-tumor immune response will result in tumor regression [1–9]. The underlying rationale is that the antigenic phenotype of cells in the tumor differs from that of normal, nonmalignant cells in the patient. The immunity to unique antigens expressed by the malignant cells, which is induced by the vaccine, would, in theory, result in the selective killing of the patient’s malignant cells only. However, relatively few antigenic determinants expressed by cancer cells are ‘tumor specific. With few exceptions, the vast majority of determinants expressed by the patient’s tumor are presented by normal, nonmalignant cells as well. MUC1 is a notable example. MUC1 is a heavily glycosylated glycoprotein associated with both normal and malignant cells. Its expression by cancer cells, specified by a mutant/dysregulated gene, is aberrant in the sense that the glycosylation pattern of the molecule is altered [10–12]. The altered glycosylation pattern renders the molecule antigenic. Other examples of dysregulated genes that specify tumor antigens include Her2/neu in breast cancer and KRAS in colon cancer [13–17]. In these instances, the density of the molecules expressed by the malignant cells is greater than that of normal cells. By contrast, cancer caused by oncogenic viruses express determinants specific to the virus. Cervical cancer caused by the human papillomavirus is a notable example. The malignant, virally infected cells express antigenic determinants specific to the papillomavirus. The products of the virally associated *E6* and *E7* genes are tumor specific [18–20]. The Epstein—Barr virus nuclear

antigen (EBNA)-1 is another example. It is expressed by Burkitt's lymphoma and nasopharyngeal carcinoma cells but not by noninfected cells in the patient [21–24]. However, few tumor antigens are the products of oncogenic viruses. Similar MUC1, they are specified by mutant or dysregulated genes in the cancer cells that differ from the homologous genes in nonmalignant cells of the patient.

The identification of tumor antigens is dependent upon their relative overabundance in cancer cells. Antibodies have been used to identify tumor antigens. Some cancer patients naturally form antibodies to antigens expressed by the their own neoplasm [25–27]. Multiple tumor antigens (MTA), including MTA1 a prostate cancer-associated antigen, have been discovered this way [28,29]. The antigenic properties of MAGE-1 derived from melanoma cells were indicated by the observation that the molecule stimulates human T cells *in vitro* [30]. The immunogenic properties of tumor antigens were further indicated by the finding that the antibodies to tumors found in cancer patients with progressively growing neoplasms reacted preferentially with the autologous tumor. The serological analysis of recombinant cDNA expression libraries (SEREX) technology takes advantage of this observation to identify new candidate tumor-associated antigens that are potential sources of vaccines for immunotherapy [31–34]. In a recent study, cancer libraries were constructed and screened using serum from a patient with prostate cancer [35]. A total of 55 genes had been previously identified, among which, nine had not been characterized. They are potential targets for immunotherapy. Among the known genes, *MTA1*, a metastasis-associated gene, was preferentially expressed in a panel of malignant tissues compared with normal tissues. *MTA1* transcripts, naturally found in normal human testes, were overexpressed in various cancer tissues.

Immunotherapy at the earliest stage of the disease provides the best chance of success

Until only recently, clinical immunotherapy trials were carried out in informed patients with advanced disease. Patients who had exhausted all forms of conventional therapy who had limited life expectancy were offered this option owing to unknown and possibly severe toxic effects of the vaccines. As cellular cancer vaccines specify predominantly normal cellular constituents and, to a lesser extent, tumor-associated antigens (TAAs), there is a danger that immunization can induce an autoimmune disease in the patient or that an oncogene or an oncogenic virus in the tumor-derived vaccine can transfer into a normal cell of the patient, generating a new malignancy. However, immuno therapy with cancer vaccines at a late stage of the patient's disease (when the tumor is large, bulky and metastatic) has little chance of success. At this point, the patient is immune suppressed. After radiation and multiple rounds of chemotherapy, the immune system is severely compromised and unable to respond vigorously to the vaccine. An additional concern is that progressively growing tumors have evolved means of avoiding the immune response. Documented tumor 'escape' mechanisms include the formation of T-regulatory and myeloid suppressor cells [36–39]. Cytotoxic T lymphocytes (CTLs) naturally recognize and destroy virally infected cells. Suppressor cells limit the extent of immune responsiveness by inhibiting the activity of CTLs after the infection has been cleared. In cancer, analogous mechanisms mediated by immune suppressor cells inhibit the activity of tumor-reactive T lymphocytes. At present, with the realization that autoimmune disease induced by cancer vaccines is rare and treatable in those uncommon occasions when it occurs and that cancer vaccines do not induce new malignancies or enhance the growth of pre-existent ones, vaccine trials are being conducted in patients at an earlier stage of the disease before immune suppression becomes predominant [38]. Patients with minimal residual disease after primary therapy are the most logical candidates. Successful vaccines can stimulate the immune system to seek out and destroy microscopic nests of cancer cells at as yet undetectable metastatic sites. At this point, the disease is too minor to be recognized by the

most sensitive diagnostic means. However, the small colonies of residual tumor can be responsible for recurrence of the patient's disease [40,41].

Active, specific immunotherapy with vaccines that target TAAs

Active, specific immunotherapy is the administration of vaccines designed to induce immunity to TAAs that have been identified as targets of immune attack. The objective is to stimulate the formation tumor-specific CTLs, predominantly CD8⁺ cells, which are directed toward antigens associated with the patient's cancer. Prostate-specific membrane antigen (PSMA) is a notable example. It has been identified as a potential therapeutic target of the immune system [42–45]. PSMA is an ideal candidate. It is expressed exclusively by both malignant and nonmalignant cells of the prostate. Patients with residual metastatic disease after radical prostatectomy can receive treatment. Other examples include HER2/neu and MUC1 expressed by breast and lung cancer cells, and the immunoglobulin idiotype associated with malignant B cells of patients with B-cell lymphoma [46–48]. Active immunotherapy has succeeded in inducing tumor-specific immune responses. Immunization of patients with B-cell lymphoma successfully induced immunity to the immunoglobulin idiotype associated with the patient's malignant B cells. However, although objective evidence of an immune response to TAAs specified by the vaccine was achieved in patients receiving tumor-specific vaccines, in many instances a beneficial clinical response did not follow. Immunization of patients with pancreatic cancer with dendritic cells (DCs) from the patient 'loaded' with telomerase peptides, as another example, gave similar mostly disappointing results [49]. One possible explanation is that antigen-negative variants in the tumor cell population failed to be recognized and destroyed. They were responsible for recurrence of the disease. Immunization with a multiepitope vaccine — a vaccine that encompasses an array to tumor antigens — might be expected to enhance its therapeutic benefits. However, immunization with vaccines that encompassed multiple tumor antigens, prepared by transfer of unfractionated mRNA from the patient's tumor [50], tumor cell lysates or by fusing the patient's tumor cells with allogeneic DCs [51] have resulted in antitumor immune responses that, in most instances, have not been accompanied by encouraging therapeutic outcomes. The vaccines attempt to induce strong CD8 and CD4 T-cell responses. The majority of vaccines recently tested in Phase I clinical trials reveal the induction of specific tumor antigen immunity; however, similar to others, clinical efficacy in the long-term remains elusive.

Advantages of a vaccine prepared by transfer of cdNA from the tumor into an immunogenic allogeneic cell line

The overriding objective of cancer immunotherapy is to administer vaccines that stimulate immunity to the broad array of antigens expressed by cells that comprise the entire tumor cell population. The likelihood that variants that fail to be included in the spectrum of antigens included in the vaccine will emerge is reduced. Administration of the vaccine after primary cancer therapy to patients with minimal residual disease provides the greatest chance of success. Therefore, a novel strategy was developed that was designed to address these issues. In a mouse breast cancer model system, the vaccine was prepared by transfer of a cDNA-expression library from SB5b cells (a breast cancer cell line derived from an adenocarcinoma that arose spontaneously in the mammary gland of a C3H/He mouse) into LM cells (a modified mouse fibroblast cell line). This approach was an application to cancer vaccine development of classic findings indicating that the genotype and phenotype of one cell type can be modified by transfer of DNA from another [52,53]. Oncogenes were first discovered by this approach, [54,55]. Preparing a vaccine by transfer of cDNA into a highly immunogenic cell line has a number of important advantages. As the cDNA expression library encompasses an array of TAAs, unlike single epitope vaccines that specify only a single antigen, CTLs, predominantly CD8⁺ cells, were generated that were directed toward the array of TAA expressed by the

malignant cell population. The possibility of recurrence of the disease from microscopic nests of metastatic malignant cells was reduced. Another advantage is that the fibroblasts, used as recipients of the cDNA, can be modified in advance of cDNA transfer to stimulate a vigorous immune response. They could be genetically modified to secrete one or more Th1 cytokines, such as IL-2, GM-CSF, IL-12 and IL-18, among others [56–59]. The secretion of immune-augmenting cytokines in the microenvironment at the immunization site serves to augment the immune response to TAAs expressed by cells in the vaccine. It can inhibit the infiltration and activity of T-regulatory cells.

Another advantage is that leukapheresis can be avoided. This costly and patient-inconvenient procedure is required to obtain sufficient numbers of DCs from the patient in order to prepare DC-based vaccines. Leukapheresis is unnecessary if the fibroblasts selected as cDNA recipients express allogeneic MHC determinants. Allogeneic determinants, which are known to be strong immune adjuvants, stimulate the uptake of the vaccine by DCs of the host, where TAAs are expressed (cross-priming). Furthermore, the expression of allogeneic MHC determinants by the cells selected as DNA recipients stimulates a vigorous immune response in the host and ensures that the vaccine, as with any other foreign tissue graft, will be rejected (the alloresponse).

Another major advantage of cDNA-based allogeneic vaccines is that they can be prepared from microgram amounts of tumor tissue [60]. A vaccine can be prepared from the tissue contained in a biopsy specimen. As the transferred cDNA integrates spontaneously into the genome of the recipient cells and replicates as the cells divide, under conventional laboratory conditions the number of vaccine cells can be readily expanded for multiple immunizations. Patients at an early stage of the disease, when tumor tissue is only available in limited amounts, can receive treatment. Stimulation of a robust anti tumor immune response directed toward multiple TAAs expressed by various cells that comprise the entire malignant-cell population can result in the elimination of microscopic nests of residual metastatic tumor, reducing the likelihood of recurrence. Our preclinical data indicate the feasibility of this approach. Immunization of mice with established neoplasms with vaccines prepared by transfer of cDNA derived from as few as 10^7 breast or squamous carcinoma cells into an allogeneic fibroblast cell line, which was then expanded in culture, resulted in prolonged and, at times, uncertain survival.

Disadvantages of preparing a vaccine by transfer of tumor-derived cdNA into a fibroblast cell line

While immunization with a vaccine prepared by transfer of tumor-derived cDNA into a highly immunogenic allogeneic cell line has a number of advantages, there are concerns. Similar to other cellular cancer vaccines, the proportion of the DNA derived from the patients' neoplasm that includes genes that specify TAAs is expected to be quite small. Various other genes ('house-keeping genes'), which specify determinants that are unrelated to the induction of tumor immunity, are in the vast majority. Thus, it is likely that a large proportion of the transfected cells do not express TAAs or they express TAAs at levels too low to induce an effective antitumor immune response.

Another concern is that, similar to other cellular vaccines, the largest proportion of the transfected cell population express genes specifying normal 'self' constituents. Conceivably, autoimmunity might develop in patients who receive the vaccine. However, in multiple preclinical studies, autoimmunity has not been observed. Tumor-free or tumor-bearing mice treated by immunization with therapeutic cDNA-based vaccines exhibited no adverse effects. They showed no evidence of autoimmune or other diseases, as indicated by an extensive microscopic examination of multiple organs and tissues of the immunized mice and by analysis of the serum for antinuclear antibodies. Of course, immunization with vaccines derived from

tumor cell extracts, peptide eluates of tumor cells, mRNAs from tumor transferred into DC or by immunization with tumor—DC hybrids are subject to the same concerns.

A further concern is that cellular vaccines derived from malignant cells might grow in the recipient, forming a tumor, or that a transforming oncogene or a defective tumor-suppressor gene might be transferred into a normal cell, provoking a neoplasm. This has not been observed. In multiple reports, tumor development following the administration of viable, nonirradiated or lethally irradiated allogeneic cellular cancer vaccines has not occurred.

Enrichment of a cdNA-based cancer vaccine for immunotherapeutic cells

As with other cellular vaccines, few cells in vaccines prepared by transfer of a cDNA expression library from the tumor into a highly immunogenic fibroblast cell line are expected to have incorporated DNA segments that specify TAAs. The vast majority expresses the products of 'housekeeping' genes, which are unrelated to the induction of tumor immunity. Therefore, a novel strategy was devised to enrich the vaccine for immunotherapeutic cells with the expectation that immunization with a vaccine that contained sufficient numbers of tumor-antigen-specific cells would generate heightened immune responses. The augmented immunotherapeutic properties of the enriched vaccines were clearly evident. Mice bearing established tumors derived from highly aggressive breast and squamous carcinoma cancer cell lines immunized with the enriched vaccines survived significantly longer than mice in various control groups, including tumor-bearing mice immunized with nonenriched vaccines.

Strategy for the enrichment of cellular tumor vaccines

Identification of immunotherapeutic (immuno^{high}) pools of transfected cells

In a mouse breast cancer model system, a cellular vaccine was prepared by transfer of an unfractionated cDNA expression library from SB5b cells (a highly aggressive breast cancer cell line derived from an adenocarcinoma that arose spontaneously in the mammary gland of a C3H/He mouse [H-2^k]), into LM cells (a mouse fibroblast cell line of C3H/He mouse origin). To ensure rejection and to augment their immunogenic properties, the cells used as cDNA recipients were modified to express allogeneic MHC class I determinants and to secrete an immune augmenting cytokine [58,59]. The vaccine was prepared from a cDNA library prepared from approximately 10^7 breast cancer cells [60].

Since few cells in the vaccine were expected to have incorporated DNA segments that included genes specifying TAAs, a unique strategy was developed to enrich the DNA-based cellular vaccine for immunotherapeutic cells [61,62]. The strategy to enrich the vaccine for immunotherapeutic cells involved dividing the transfected cell population into a number of small pools. If the starting inoculums are sufficiently small, then, randomly, some will contain greater numbers of immunotherapeutic cells than others. Pools with greater numbers of immunotherapeutic cells can be identified by comparing the cells' relative immunogenic properties against the tumor in mice highly susceptible to the growth of the neoplasm. To test this strategy, 1×10^3 transfected cells were added to each of ten replicate wells of a 96-well plate. Cells from the individual pools were allowed to increase in number to approximately 5×10^7 , by successive transfer to progressively larger cell culture plates and then cell culture flasks. Afterward, half of each of the expanded cell populations was collected and maintained (frozen/viable) for later recovery. The remaining portion was used to immunize naive C3H/He mice. After immunization, spleen cells from the mice were tested by two independent means (ELISPOT IFN- γ -release for responding T cells and ⁵¹Cr-release cytotoxicity assays for CTLs) to identify pools that stimulated T-cell-mediated immunity toward the breast cancer cells to the greatest extent. These pools were designated as immuno^{high}. Pools that stimulated immunity to the breast cancer cells to the least extent were also identified for later use as

controls. These pools were designated immuno^{low}. Cells from immuno^{high} and immuno^{low} pools were recovered from frozen stocks, re-established in culture, divided into a succession of small pools and subjected to additional rounds of positive or negative immune selection. This strategy is outlined in Figure 1.

Results indicated that the immunogenic properties of transfected cells from each of the individual pools were not the same. Cells from one subpool (SP; SP6–6) stimulated immunity toward the breast cancer cells to the greatest (SP6–6 = immuno^{high}). In an analogous manner, cells from SP10–4 stimulated breast cancer immunity to the least extent (SP10–4 = immuno^{low}). Frozen/viable cells from these pools were recovered and the procedure was repeated for additional rounds of positive or negative immune selection. 1×10^3 transfected cells were used as the starting inoculums in each instance.

Enhanced T-cell responses toward breast cancer cells were generated in mice following immunization with cellular vaccines enriched for immunotherapeutic cells

ELISPOT IFN- γ assays were used to compare the relative immunogenic properties of transfected cells from the immuno^{high} pools after one (1°), two (2°), three (3°) or four (4°) rounds of positive immune selection. Naive C3H/He mice were injected subcutaneously twice at weekly intervals with equivalent numbers of cells from each of the immuno^{high} pools. As controls, the mice were injected according to the same schedule with cells after four rounds of negative immune selection (immuno^{low} pool [4°] or with cells from the nonenriched master pool. After 1 week following the second injection, varying numbers of spleen cells (from 2×10^6 serial twofold dilutions) from each group were coincubated for 18 h with breast cancer cells. Afterward, the relative numbers of responding T cells were determined by ELISPOT IFN- γ assays.

The results revealed a progressive increase in the number of responding splenic T cells after immunization with vaccine after two, three or four rounds of positive immune selection. The highest number of responding T cells were present in spleen cell suspensions from mice immunized with cells from the immuno^{high} pool (4°) ($p < 001$) compared with the number of responding cells from mice immunized with cells from the nonenriched master pool. Fewer numbers were found if the mice were immunized with cells from immuno^{high} pools after one, two or three rounds of positive selection. The least number of responding T cells were from mice immunized with cells from the immuno^{low} pool (4°).

Survival of mice with breast cancer treated by immunization with cells from the enriched vaccine

The results of the prior assays revealed the enhanced immunogenic properties of cells from the enriched vaccine. To determine if the heightened immunogenic properties of the vaccines observed in the *in vitro* assays were reflected by enhanced therapeutic responses *in vivo*, the survival of mice with established tumors derived from the breast cancer cells treated solely by immunization with cells from the immuno^{high} pool (2°) was compared with that of tumor-bearing mice treated with cells from nonenriched vaccines.

Tumors were first established in C3H/He mice following a subcutaneous injection of 0.5×10^6 viable breast cancer cells into the left flank. After 6 days, the tumor-bearing mice received the first of two weekly subcutaneous injections in the opposite flank of 4×10^6 cells from the immuno^{high} pool (2°). As controls, the same procedure was followed except that equivalent numbers of cells from the immuno^{low} pool (2°) or the nonenriched master pool (LM-IL-2K^b/

cSB5b) were substituted for cells from the immuno^{high} pool (2°). The results, presented in Figure 2, indicated that all of the tumor-bearing mice treated by immunization with cells from the immuno^{high} pool survived more than 55 days without evident disease; they appeared to have rejected the breast cancer cells. Under similar conditions, none of the tumor-bearing mice treated by immunization with cells from the immuno^{low} pool or untreated mice injected with the breast cancer cells alone survived. The animals died from progressive tumor growth ($p < 0.01$ for differences in the period of survival of mice in the various groups versus mice treated by immunization with cells from the immuno^{high} pool). The greatest therapeutic benefit was clearly evident in the group of tumor-bearing mice treated by immunization with cells from the enriched vaccine.

The immunity to breast cancer induced by immunization with cells from the enriched vaccine was long-term. Surviving mice were injected a second time with the breast cancer cells 55 days after the first. As previously mentioned, 100% of the mice survived. Under similar circumstances, none of the mice in the control group injected with the breast cancer cells alone survived. They died from progressive tumor growth ($p < 0.001$ for differences in survival of mice in the two groups).

The enhanced immunotherapeutic properties of the enriched vaccines were clearly evident.

Conclusion

- The antigenic phenotype of cells in the tumor differs from that of normal, nonmalignant cells of the patient;
- Cellular cancer vaccines are designed to induce immunity to unique antigens expressed by the malignant cells;
- Vaccines are prepared by transfer of extracts from the patient's tumor into DCs (antigen-presenting cells) from the patient may contain an insufficient number of cells that express tumor antigens;
- A unique enrichment strategy described in this review results in an increase in the proportion of cells in the vaccine that express tumor antigens with a corresponding augmentation of the vaccine's therapeutic benefits

Future perspective

Effective cancer vaccines are needed as adjuncts to conventional cancer therapy. Since the need is so great, the ideal vaccine should be effective and able to be readily prepared at low cost.

A cellular vaccine prepared by transfer of a cDNA expression library into a highly immunogenic allogeneic cell line has a number of unique advantages. The cell line chosen as the recipient of cDNA from the tumor can be readily transfected under conventional laboratory conditions. Specialized equipment or technically challenging procedures are not required. The recipient cells can be modified in advance of cDNA transfer to augment their nonspecific immunogenic properties. In this instance, the cells were modified to secrete IL-2 — a Th1 cytokine — and to express foreign (allogeneic) MHC class I determinants. A major advantage of this is that as the transferred cDNA spontaneously integrates into the genome of the recipient cells, replicates as the cells divide and is expressed, the vaccine could be prepared from microgram amounts of amounts of tumor tissue. Sufficient DNA to prepare the vaccine was obtained from tumors as small as 4 mm. The ability to generate an effective vaccine from such small neoplasms provides an opportunity to prepare vaccines for patients with small primary

neoplasms who are at a high risk of recurrence from microscopic tumors remaining after completion of conventional therapy.

However, as with other cellular cancer vaccines, only a small proportion of the determinants expressed by the transfected cells were tumor specific. A unique strategy that has general applicability was developed to enrich the vaccine for immunotherapeutic cells. The enriched vaccine expressed an array of multiple antigens associated with the tumor cancer cells. The novel enrichment strategy generated a vaccine with enhanced immunotherapeutic properties.

The strategy described here raises the possibility that an analogous approach could be used to generate cellular vaccines of enhanced effectiveness, which could become part of the overall management of cancer patients at an early stage of the disease.

Executive summary

- The vast majority of the constituents of tumor cells express the products of widely shared normal 'housekeeping' genes that are not tumor specific.
- Cellular cancer vaccines prepared by the transfer of tumor cell extracts into dendritic cells may fail to control tumor growth because they contain an insufficient number of cells that express tumor antigens and induce antitumor immunity directed toward the patient's cancer.
- Cancer vaccines prepared by transfer of a cDNA expression library into a nonmalignant allogeneic cell line have a number of practical advantages; however, similar to other vaccines, the majority of cells in the vaccine fail to express tumor-specific antigens.
- A unique strategy was developed to enrich the cDNA-based vaccine for immunotherapeutic cells. Mice with established tumors derived from an adenocarcinoma that arose spontaneously in the mammary gland of a C3H/He mouse treated solely by immunization with the enriched vaccine survived significantly longer than mice in various control groups. Some of the immunized tumor-bearing mice appeared to have rejected the malignant cells; they survived indefinitely.

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Figure 1. Strategy for enrichment of a cellular cancer vaccine using immunotherapeutic cells
 A cDNA expression library from SB5b cells, a breast cancer cell line, was transfected into LM fibroblasts. The transfected cell population (10^3) was divided into a number of small pools. Cells in the individual pools were allowed to increase in number to approximately 10^7 , by transfer to progressively larger cell culture flasks. Afterward, half of the cells from the individual pools was maintained (frozen/viable) for later recovery. The remaining half was used to immunize C3H/He mice, which were highly susceptible to the growth of the breast cancer cells. Analyzing spleen cells from the immunized mice by two independent means identified pools containing greater numbers of cells that expressed tumor antigens. Highly immunogenic pools were identified and subjected to multiple rounds of enrichment.

Figure 2. Immunity to breast cancer in mice immunized cells from immuno^{high} subpool of transfected cells

C3H/He mice were injected into the mammary fat pad with 2×10^5 breast cancer cells. After 6 days, the tumor-bearing mice received the first of two weekly subcutaneous injections of 4×10^6 cells from the immuno^{high} (2°) pool (SP6-6). As controls, the same procedure was followed except that cells from the immuno^{high} (1°) pool (SP6), the immuno^{low} (2°) pool (SP10-4), or cells from the nonenriched master pool (LM-IL-2Kb/cSB5b) were substituted for cells from the immuno^{high} (2°) pool. As a specificity control, one group of tumor-bearing mice was injected with fibroblasts transfected with a cDNA library from B16F1 melanoma cells (LM-IL-2Kb/cB16F1). $p < 0.01$ for the differences in survival of mice with breast cancer treated by immunization with cells from the immuno^{high} (2°) pool and mice in any of the other groups.